

A *Chlamydomonas* Outer Arm Dynein Mutant with a Truncated β Heavy Chain

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Abstract. A new allele of the *Chlamydomonas oda4* flagellar mutant (*oda4-s7*) possessing abnormal outer dynein arms was isolated. Unlike the previously described *oda4* axoneme lacking all three (α , β , and γ) outer-arm dynein heavy chains, the *oda4-s7* axoneme contains the α and γ heavy chains and a novel peptide with a molecular mass of ~ 160 kD. The peptide reacts with a mAb (18 β B) that recognizes an epitope on the NH₂-terminal part of the β heavy chain. These observations indicate that this mutant has a truncated β heavy chain, and that the NH₂-terminal part of the β heavy chain is important for the stable assembly of the outer arms. In averaged electron microscopic images of outer arms from cross sections of axonemes, the mutant outer arm lacks its mid-portion, producing

a forked appearance. Together with our previous finding that the mutant *oda11* lacks the α heavy chain and the outermost portion of the arm (Sakakibara, H., D. R. Mitchell, and R. Kamiya. 1991. *J. Cell Biol.* 113:615–622), this result defines the approximate locations of the three outer arm heavy chains in the axonemal cross section. The swimming velocity of *oda4-s7* is $65 \pm 8 \mu\text{m/s}$, close to that of *oda4* which lacks the entire outer arm ($62 \pm 8 \mu\text{m/s}$) but significantly lower than the velocities of wild type ($194 \pm 23 \mu\text{m/s}$) and *oda11* ($119 \pm 17 \mu\text{m/s}$). Thus, the lack of the β heavy chain impairs outer-arm function more seriously than does the lack of the α heavy chain, suggesting that the α and β heavy chains play different roles in outer arm function.

THE inner and outer dynein arms of eukaryotic cilia and flagella are mechanochemical transducers that cause sliding movements between outer doublets (see Gibbons, 1981). Of the two kinds of arms, the outer arm has been the most extensively studied. The outer arm contains two or three ATPase heavy chains and, when isolated from the axoneme, takes on a bouquet-like structure in which two or three globular heads are bundled at their base (Johnson and Wall, 1983; Witman et al., 1983; Goodenough and Heuser, 1984; Sale et al., 1985). Because the number of the heads equals that of the heavy chain species present in the outer arm, each head appears to be composed of a distinct heavy chain. It is not known, however, how the bouquet-like outer arm is folded in the axoneme, or what function is performed by each heavy chain.

Chlamydomonas outer arm dynein is composed of three heavy chains (named α , β , and γ) with molecular masses >400 kD, two intermediate-sized subunits of 69 and 78 kD, and several light chains (Piperno and Luck, 1979; Pfister et al., 1982; King and Witman, 1988a,b). Several types of outer-arm-deficient mutants have been isolated in this organism, including ten motile strains (*odal-oda10*) lacking the entire outer arm (Kamiya and Okamoto, 1985; Mitchell and Rosenbaum, 1985; Kamiya, 1988) and one (*oda11*) that lacks the α heavy chain and a 16-kD light chain but retains the

other components (Sakakibara et al., 1991). The mutant *oda11* swims slower than wild type but faster than mutants lacking the entire outer arm. This indicates that outer dynein arms without the α heavy chain retain some function. The *oda11* mutation has been shown to be closely linked with the structural gene for the α heavy chain (Sakakibara et al., 1991). On the other hand, the outer arm-less mutant, *oda4*, has been suggested to have a defect in the structural gene for the β heavy chain (Luck and Piperno, 1989; Ranum et al., 1988). The mutant *sup_{pf1}*, isolated as a suppressor of the paralyzed phenotype of mutants defective in the central-pair/radial spoke system, has been shown to have a β heavy chain with a slightly higher electrophoretic mobility (Huang et al., 1982). This mutant and *oda4* appear to be allelic to each other (Luck and Piperno, 1989). In the absence of the central pair or radial spoke mutation background, *sup_{pf1}* flagella beat more slowly than wild-type flagella but slightly faster than *oda* flagella (Brokaw and Kamiya, 1987). Although these properties of *sup_{pf1}* suggest that the β heavy chain plays a key function in wild-type flagellar movement, it is not certain whether properties of the *sup_{pf1}* derive from an alteration that simply renders the β heavy chain unable to generate force, or from one that somehow affects the functioning of the entire arm.

In this report, we show that the outer dynein arm of a

newly isolated *oda4* allele, *oda4-s7*, contains a truncated β heavy chain of about one-third the normal size, as well as normal α and γ heavy chains. This mutant swims only slightly faster than the mutant *oda4* which lacks the entire outer arm, but much more slowly than *oda11* lacking the α heavy chain. Hence, lack of the β heavy chain impairs outer arm function more seriously than does lack of the α heavy chain. This finding indicates that the α and β heavy chains have different roles in the function of the outer arm.

Materials and Methods

Strains

The following *Chlamydomonas reinhardtii* strains were used: 137c (wild type; mating type + and -), *oda4* lacking the entire outer arm (Kamiya, 1988), *oda11* lacking the α heavy chain and 16-kD light chain of the outer arm (Sakakibara et al., 1991), *pfl8* lacking the central pair (Witman et al., 1972), and the newly isolated mutant *oda4-s7*.

Mutant Isolation

Mutagenesis of cells and separation of slow-swimming cells were carried out as described previously (Sakakibara et al., 1991). Cells that were judged to be slow swimmers were examined for the composition of flagellar dynein heavy chains by SDS-PAGE. Those that had abnormal SDS-PAGE patterns were saved and mated with the wild type; a daughter cell of the original phenotype was mated with the wild type again and progenies of both mating types were saved.

Immunoblotting Assay

Polypeptides of axonemes separated by SDS-PAGE on a 3–8-M urea/3–5% acrylamide gradient gel were transferred to a nitrocellulose sheet using a semi-dry system (Khyse-Anderson, 1984). The sheet was reacted with the mAb 18 β B (King et al., 1985) and an alkaline phosphatase-conjugated secondary antibody (Jackson Immuno Research Labs, Inc., West Grove, PA). The reacted bands were visualized by color development with nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate (Sigma Immunochemicals, St. Louis, MO).

Isolation of Axonemes and Fractionation of Dynein

Culture of cells, isolation of flagella, preparation of crude dynein extracts, and sucrose density gradient centrifugation have been described previously (Sakakibara and Kamiya, 1989; Sakakibara et al., 1991). These procedures were essentially after King et al. (1986).

ATPase Assay

ATPase activities of the fractionated dynein were measured by a malachite green method as described by Kodama et al. (1986). The ATP-hydrolyzing reaction was allowed to proceed at 25°C in the presence of 10 mM Tris-HCl, pH 7.5, 5 mM MgSO₄, 0.5 mM EDTA, and 1 mM ATP.

Electron Microscopy

Axonemes were fixed overnight at 4°C with 2% glutaraldehyde in the presence of 1% tannic acid, in 50 mM sodium cacodylate, pH 7.0. After postfixation with 1% OsO₄ for 1 h, the sample was stained en bloc with 0.5% uranyl acetate for 1 h at 4°C, dehydrated through a series of ethanol solutions at room temperature, and embedded in Epon 812. Golden thin sections were cut and double-stained with aqueous solutions of 7.5% uranyl acetate and 0.4% lead citrate. Specimens were viewed in a JEM100C microscope.

To examine the shape of outer dynein arms in mutants, images of outer-doublet microtubules in cross sections of axonemes were averaged using an Excell image processor (Avionics, Tokyo) as previously described (Kamiya et al., 1990; Sakakibara et al., 1991).

Motility Assessment

The swimming velocities of the mutant and wild-type cells were measured

using a dark-field microscope at a total magnification of 100, a video recording system, and a personal computer. A red filter (cut-off wavelength: 630 nm) was placed under the condenser lens to facilitate the measurement by suppressing the cells' response to light; when viewed using white light, *Chlamydomonas* cells frequently display irregular swimming paths, which make the measurement extremely difficult. 50 samples were measured to obtain the average velocity of a given sample.

Flagellar beat frequencies of swimming cells were measured by the fast Fourier transform (FFT) method described by Kamiya and Hasegawa (1987). This method analyzes the light intensity fluctuation in the microscope image of a population of swimming cells and yields an average beat frequency within 10–20 s. About 10 independent measurements were performed for each strain. Beat frequency in demembrated cell models were measured as described previously (Sakakibara and Kamiya, 1989). All the motility measurements were carried out at 25°C. To evaluate the average number of outer dynein arms present in the mutant axonemes in a particular sample, cells that were assayed for motility were demembrated, fixed, and processed for electron microscope observations.

Other Methods

SDS-PAGE was carried out by the method of Laemmli (1970) as modified by Jarvik and Rosenbaum (1980). The gel was composed of a 3–5% acrylamide gradient and a 3–8 M urea gradient. To analyze the intermediate and low molecular weight dynein components, the samples were also run on 20% acrylamide slab gels. All the gels were silver-stained (Merrill et al., 1981).

Protein concentrations of the axoneme and the crude extract were determined by the method of Bradford (1976).

Tetrads between *oda4-s7* and other mutants were analyzed using standard techniques (Levine and Ebersold, 1960).

Results

Lack of the β Heavy Chain in an *oda4* Allele, *oda4-s7*

The new mutant *oda4-s7* was isolated as a mutant having a motility phenotype similar to that of the mutants *oda1-oda10*, which lack the entire outer dynein arm. This mutant appeared to be an allele of *oda4* because it did not segregate with the *oda4* mutant in 100 tetrads, and temporary dikaryons between these strains did not rescue the slow-swimming phenotype. SDS-PAGE patterns of axonemes showed that *oda4-s7* axoneme lacks the β heavy chain but retains the α and γ heavy chains, whereas the normal *oda4* axoneme lacks all three dynein heavy chains (Fig. 1). The α and γ heavy chain bands in this mutant are less intense than in the wild type. As will be shown later, this is because the total number of outer arms per axoneme is reduced in this mutant.

Western Blot Analysis

We suspected that the *oda4-s7* axoneme might have a truncated β dynein heavy chain, because mutations at the *oda4* locus usually result in loss of the entire outer arm (Kamiya, 1988), and because the NH₂-terminal portion of the β heavy chain has been shown to interact with the intermediate chains which are believed to be important in attaching the heavy chain to the A-tubule of the outer doublet microtubule (Mitchell and Rosenbaum, 1986; King et al., 1991). If the NH₂-terminal portion of the β heavy chain was preserved in *oda4-s7*, it would explain why this mutant retains the α and γ heavy chains. We therefore examined *oda4-s7* axonemes by Western blot analysis with the 18 β B mAb which reacts with an epitope in the NH₂-terminal region of the β heavy chain (King et al., 1988a). (King and Witman [1989] reported that the 18 β B epitope and the intermediate chains were located near the COOH terminus of the β heavy chain. However, recent molecular genetic data indicate that the

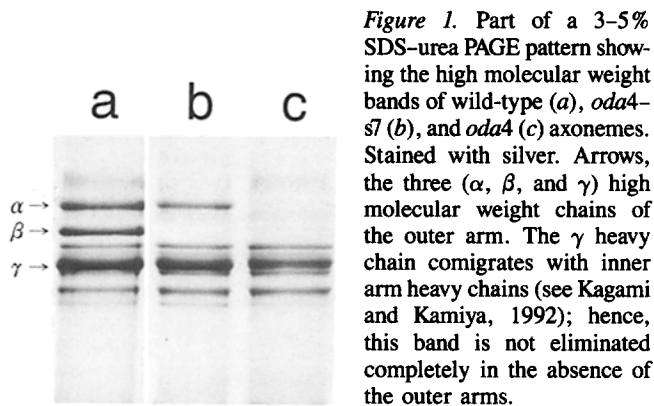


Figure 1. Part of a 3–5% SDS-urea PAGE pattern showing the high molecular weight bands of wild-type (a), *oda4-s7* (b), and *oda4* (c) axonemes. Stained with silver. Arrows, the three (α , β , and γ) high molecular weight chains of the outer arm. The γ heavy chain comigrates with inner arm heavy chains (see Kagami and Kamiya, 1992); hence, this band is not eliminated completely in the absence of the outer arms.

polarity of the map should be reversed [Fig. 2 A] [King and Witman, manuscript in preparation].) Fig. 2 B demonstrates that *oda4-s7* axonemes contain a novel peptide of ~ 160 kD that is absent from the wild-type and *oda4* axonemes. Importantly, this peptide reacted with the 18 β B antibody. This finding indicates that the axoneme of *oda4-s7* has a truncated form of the β heavy chain.

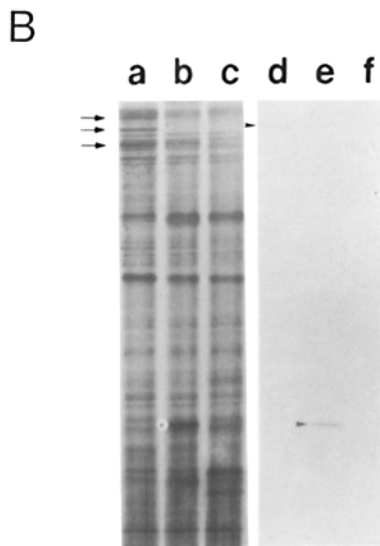
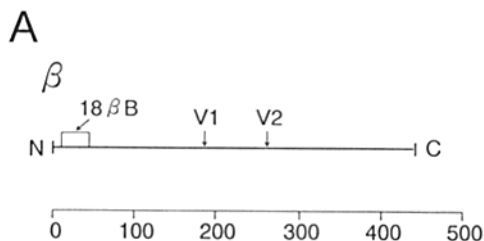


Figure 2. Immunoblot of axonemes. (A) Revised map of the β heavy chain showing the site recognized by the 18 β B mAb relative to the V1 and V2 cleavage sites. The scale of the diagram is shown in kilodaltons (King and Witman, 1988a). (B) (a, b, c) 3–5% SDS-urea polyacrylamide gel of wild-type (a and d), *oda4-s7* (b and e), and *oda4* (c and f) axonemes stained with silver; d, e, and f, lanes identical to a, b, and c probed with the 18 β B antibody. Arrows, the three (α , β , γ) high molecular weight chains of the outer arm. Arrowheads, bands that reacted with the antibody. Asterisk, novel 160-kD polypeptide found only in the *oda4-s7* axoneme.

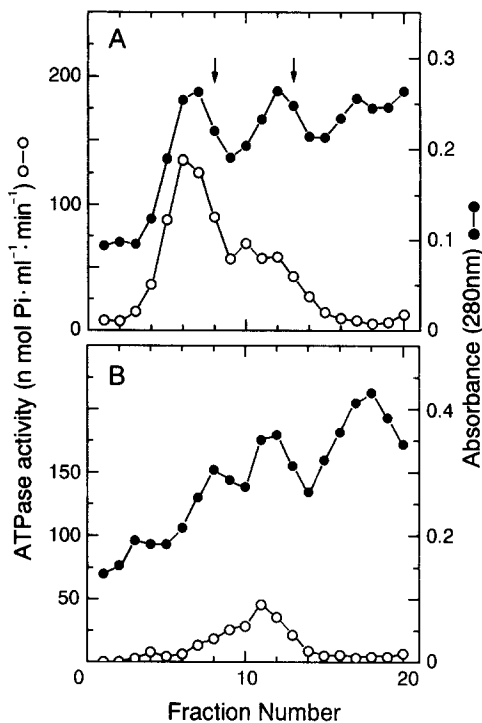


Figure 3. Sucrose density gradient centrifugation profiles of 0.6 M KCl extracts from wild-type (A) and *oda4-s7* (B) axonemes. The bottom of the gradient is at left. (●), A_{280} protein absorbance of the fractions. (○) Mg^{2+} -dependent ATPase activity expressed as nmole Pi/ml/min. Arrows indicate the positions of the sedimentation peaks of bovine thyroglobulin (19.3 S) and catalase (11.3 S).

Sucrose Density Gradient Centrifugation

For further analysis of dynein components in the mutant, the axoneme was extracted with 0.6 M KCl and the extract was subjected to a 5–20% sucrose density gradient centrifugation (Fig. 3). As has been reported previously (Watanabe and Flavin, 1973; Piperno and Luck, 1979; Pfister and Witman, 1984), major Mg-ATPase activities in the wild-type extract appeared in two peaks: one that contains the α and β heavy chains at 21–22 S (fractions 6 and 7) and the other that contains the γ heavy chain at 13–15 S (fractions 10–12) (Fig. 4). With the *oda4-s7* axoneme extract, no ATPase peak appeared at 21–22 S (Fig. 3). SDS-PAGE of each fraction indicated that the α heavy chain appeared at about ~ 18 –19 S (fractions 8–9) instead of 21–22 S (Fig. 4). The ATPase activity of the 18–19-S region in the *oda4-s7* extract was very low, appearing only as a shoulder of the 13–15-S peak (Fig. 3). The 18–19-S fractions also contain the novel 160-kD chain and the two outer arm intermediate chains, known to be associated with the β heavy chain (Pfister and Witman, 1984; Mitchell and Rosenbaum, 1986) (Fig. 5). As in the wild-type extract, the 13–15-S peak contains the γ heavy chain and several inner arm heavy chains.

To examine the protein compositions in a lower molecular weight range, we also performed SDS-PAGE using 20% acrylamide gels. In the wild-type outer arm, a 16-kD light chain is associated with the α heavy chain and a 19-kD light chain with the β heavy chain. In addition, six light chains of 8, 8, 11, 14, 14, and 20 kD are complexed with the intermediate chains (69 and 78 kD) and attached to the NH₂-terminal portion of the β heavy chain (Pfister et al., 1982;

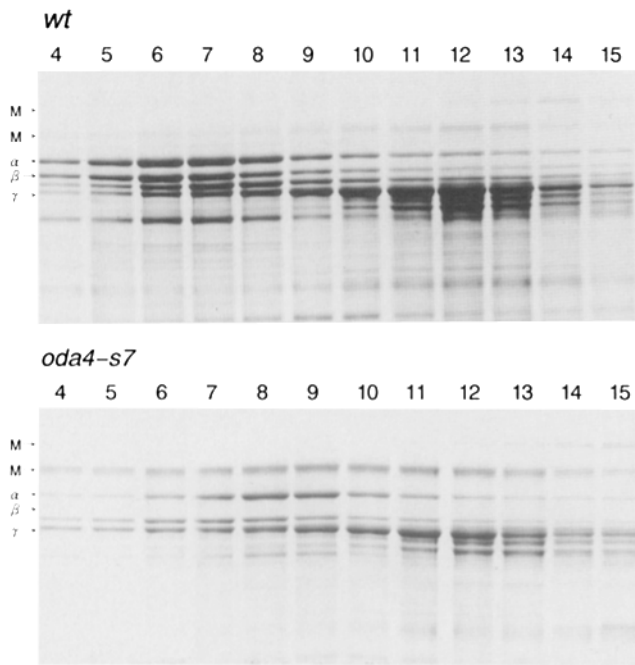


Figure 4. SDS-PAGE patterns of fractions from the sucrose density gradient centrifugation shown in Fig. 3. Only high molecular weight regions of SDS-PAGE patterns are shown for comparison between the two strains. An equal volume of each fraction was loaded on each lane. The number above each lane is that for the fraction in Fig. 3. Arrows, the three (α , β , and γ) high molecular weight chains of the outer arm. The bands marked *M* represent residual membrane proteins. A band present below the γ heavy chain in lanes 5–9 of wild-type pattern is the α heavy chain proteolytic fragment known as band *II* (Pfister and Witman, 1984; King and Witman, 1988a). Stained with silver.

Pfister and Witman, 1984; Mitchell and Rosenbaum, 1986; King and Witman, 1990). In the *oda4-s7* axoneme, all of the light chains and the intermediate chains known to be associated with the β heavy chain were present in the 18–19-S fractions (Fig. 6). Two other light chains of 18 and 22 kD normally associated with the γ heavy chain were also present in the 13–15-S fractions. Thus none of the dynein subunits other than the β heavy chain is impaired in this mutant.

Electron Microscope Observation on In Situ Outer Arms

In a previous study on *odal1*, we demonstrated that the α heavy chain makes up the outermost appendage of the outer dynein arm. To determine where the β heavy chain is located, we examined electron micrographs of cross sections of axonemes. We found that the average number of outer dynein arms in cross sections of *oda4-s7* axonemes was fewer than that in wild type. Measurements in 50 cross sections indicated that the average number in the mutant axoneme was $5.6 (\pm 1.1)$ (Fig. 7), i.e., $\sim 70\%$ of the wild-type value of 8 (Huang et al., 1979; Hoops and Witman, 1983). Similar results were obtained with two other independent preparations. *oda4-s7* thus appears to have an impaired ability to assemble outer dynein arms on the outer doublets.

Fig. 8 shows cross section electron micrographs of the axonemes of wild type (*A*) and *oda4-s7* (*B*). The mid-portion of the outer arm of *oda4-s7* looked less electron dense than

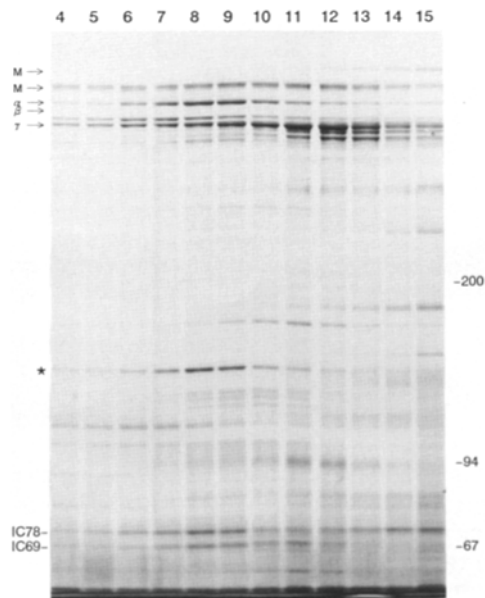


Figure 5. SDS-PAGE pattern of fractions from the sucrose density gradient centrifugation of *oda4-s7* axoneme extract showing the distribution of intermediate-sized chains. Arrows, the three (α , β , and γ) high molecular weight chains of the outer arm. Asterisk, the novel 160-kD chain. *M*, membrane protein band. *IC78* and *IC69*, 78- and 69-kD intermediate chains. Bars and numbers at right, positions of molecular weight standards with molecular mass in kilodaltons. Stained with silver.

that of wild type. To examine the structural defect in more detail, we averaged the eight outer doublet images (Fig. 8, lower rows) from selected cross sections of *oda4-s7* axonemes that had a normal number (i.e., eight) of outer arms. The averaged image of the *oda4-s7* outer arm is weak at its mid-portion, giving rise to a forked appearance. This suggests that a major part of the β heavy chain, the COOH-terminal part missing in *oda4-s7*, is located in the mid-portion of the outer arm.

To obtain further information on the heavy chain location, we constructed a double mutant, *oda4-s7xodal1*, in the hope

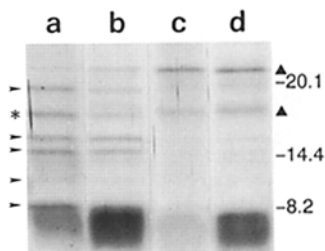


Figure 6. Low molecular weight chains separated in 20% SDS-polyacrylamide gels. Fraction 7 (21S) of wild type (*a*), fraction 8 (19S) of *oda4-s7* (*b*), fraction 11 (13S) of wild type (*c*) and fraction 11 of *oda4-s7* (*d*) are compared. Arrowheads indicate the light chains (8, 11, 14, 16, and 20 kD) which make up

the intermediate chain–light chain complex located at the base of the 18 S dynein (King and Witman, 1990; Pfister et al., 1982; Pfister and Witman, 1984; Mitchell and Rosenbaum, 1986). Asterisk indicates the 19-kD light chain (17.5 kD) also normally associated with the β heavy chain, but whose location relative to that chain's structural domains has not been determined. (\blacktriangle) The light chains (18 and 22 kD) normally associated with the γ heavy chain. Only a portion where the light chain bands appear is shown. Bars and numbers at right, positions of standards with molecular mass in kilodaltons. Stained with silver.

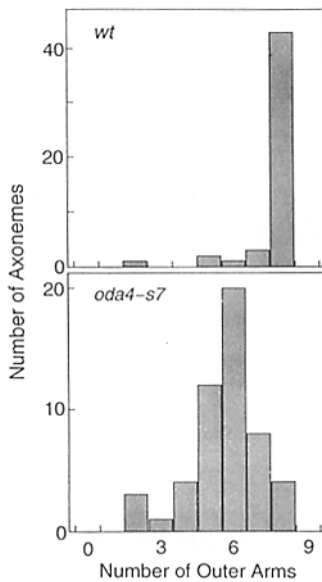


Figure 7. Histogram of the number of outer arms present in cross section images of the *oda4-s7* and wild-type axonemes. The average number (SD) is 5.6 (1.1) in *oda4-s7* and 7.6 (1.0) in wild type.

that the removal of the α heavy chain from the *oda4-s7* would result in clearer localization of the β heavy chain. The double mutant *oda4-s7xodal1* had still fewer outer arms per axonemal cross section (about four), and very few cross sections showed eight arms. For the image averaging we therefore used only four to seven outer doublet images that had outer arms attached. Fig. 9 compares the typical images of *wt*(A), *oda4-s7* (B), *odal1*(C), and *oda4-s7xodal1*(D). In the 10 micrographs analyzed, the averaged images of the *oda4-s7xodal1* outer arms were always significantly smaller than that of *odal1*. Because the outer arm in the double mutant has the defects in the α and β heavy chains combined, the image of the outer arms in the double mutant further supports the view that the β heavy chain contributes to the mid-portion of the outer arm, and that the γ heavy chain makes up the remaining portion, i.e., the inner lobe of the outer arm.

Swimming Velocity and Flagellar Beat Frequency

An important question about the *oda4-s7* outer arm dynein is whether it is functionally active. To answer this question, we compared the swimming velocity and flagellar beat frequency between *oda4-s7* and *oda4*. As the histograms in Fig. 10 show, the mean swimming velocity of *oda4-s7* ($64.9 \pm 7.7 \mu\text{m/s}$) was close to that of *oda4* ($61.7 \pm 8.2 \mu\text{m/s}$), but much lower than those of the wild type ($194.3 \pm 23.3 \mu\text{m/s}$) and *odal1* ($118.7 \pm 17.0 \mu\text{m/s}$). However, the swimming velocity of *oda4-s7* was slightly higher than that of *oda4*. Also, the median flagellar beat frequency of *oda4-s7* ($30 \pm 3 \text{ Hz}$) measured with a large population of cells was slightly higher than that of *oda4* ($26 \pm 3 \text{ Hz}$). The differences in the swimming velocity and beat frequency between the two strains, albeit very small, were consistently observed in two other independent experiments. The difference in the beat frequency was also observed in demembranated, reactivated cell models; median beat frequency at 1 mM ATP was 33 Hz in *oda4-s7* and 28 Hz in *oda4*. These results indicate that the outer arm dynein of *oda4-s7* with a truncated β heavy chain has lost nearly all of its function.

Lack of a Suppressor Activity

A mutation in the β heavy chain, *sup_{pf1}*, has been shown to have an ability to rescue the paralyzed-flagella phenotype caused by the lack of the central pair or radial spokes (Huang et al., 1982); thus the double mutant between *sup_{pf1}* and *pf18* (missing the central pair) or *pf14* (missing the radial spokes) can swim slowly. It is not known whether this suppressor activity results from a particular alteration of the β heavy chain function in this mutant or from the absence of β heavy chain function in general. We therefore constructed double mutants *oda4-s7xpf18* and *oda4-s7xpf14* to address this question. Neither double mutant regained motility; both displayed phenotypes similar to the parent *pf* mutants. Hence, the suppressor activity possessed by *sup_{pf1}* appears not to be due to a simple loss of β heavy chain function.

Discussion

Mutation in the β Heavy Chain

We have shown that the outer dynein arm of *oda4-s7* lacks the β heavy chain (>400 kD; King and Witman, 1987; Mitchell, D. R., personal communication) but instead contains a novel polypeptide of 160 kD. Western blot analysis of *oda4-s7* axonemes revealed that this novel polypeptide contains an epitope normally located in the NH₂-terminal portion of the β heavy chain. In addition, our Northern blot analysis indicates that the β heavy chain messenger in *oda4-s7* is of the expected size ($\sim 14 \text{ kb}$) (Wilkerson and Witman, unpublished results); therefore, it could not contain a sizable deletion. It is thus likely that *oda4-s7* has a mutation in the structural gene for the β heavy chain that results in a COOH-terminal truncation of the product. This mutation also may affect the amount of β heavy chain produced, or the efficiency of outer arm assembly, because the number of outer arms in this mutant is reduced to $\sim 70\%$ of the normal value.

The 160-kD β chain of *oda4-s7* apparently has lost the ATPase catalytic site, which is located at the V1 site $\sim 180 \text{ kD}$ from the NH₂ terminus of the β heavy chain (Fig. 2 A) (King and Witman, 1987; Mitchell, D. R., personal communication). In accordance with this view, sucrose density gradient profiles of the high-salt extract of *oda4-s7* axonemes revealed a very low ATPase activity in the 19–20-S fractions which contained the α heavy chain and the small β heavy chain; this ATPase activity was significantly lower than that of the corresponding 21–22-S peak fractions of the wild-type extract. Since the β heavy chain has been shown to account for most of the ATPase activity of the entire 21–22-S particle in the wild-type extract (Pfister and Witman, 1984), the lower activity in the *oda4-s7* extract probably is due to loss of the ATP hydrolytic site of the β heavy chain.

It is of interest that *oda4-s7* retains the 19-kD light chain, the location of which in the β subunit has not been determined. This result indicates that the 19-kD light chain is associated with the NH₂-terminal one third of the β heavy chain.

Implications for the Outer Arm Structure

In cross section electron micrographs, the *oda4-s7* outer dynein arm lacked its mid-portion, producing a forked ap-

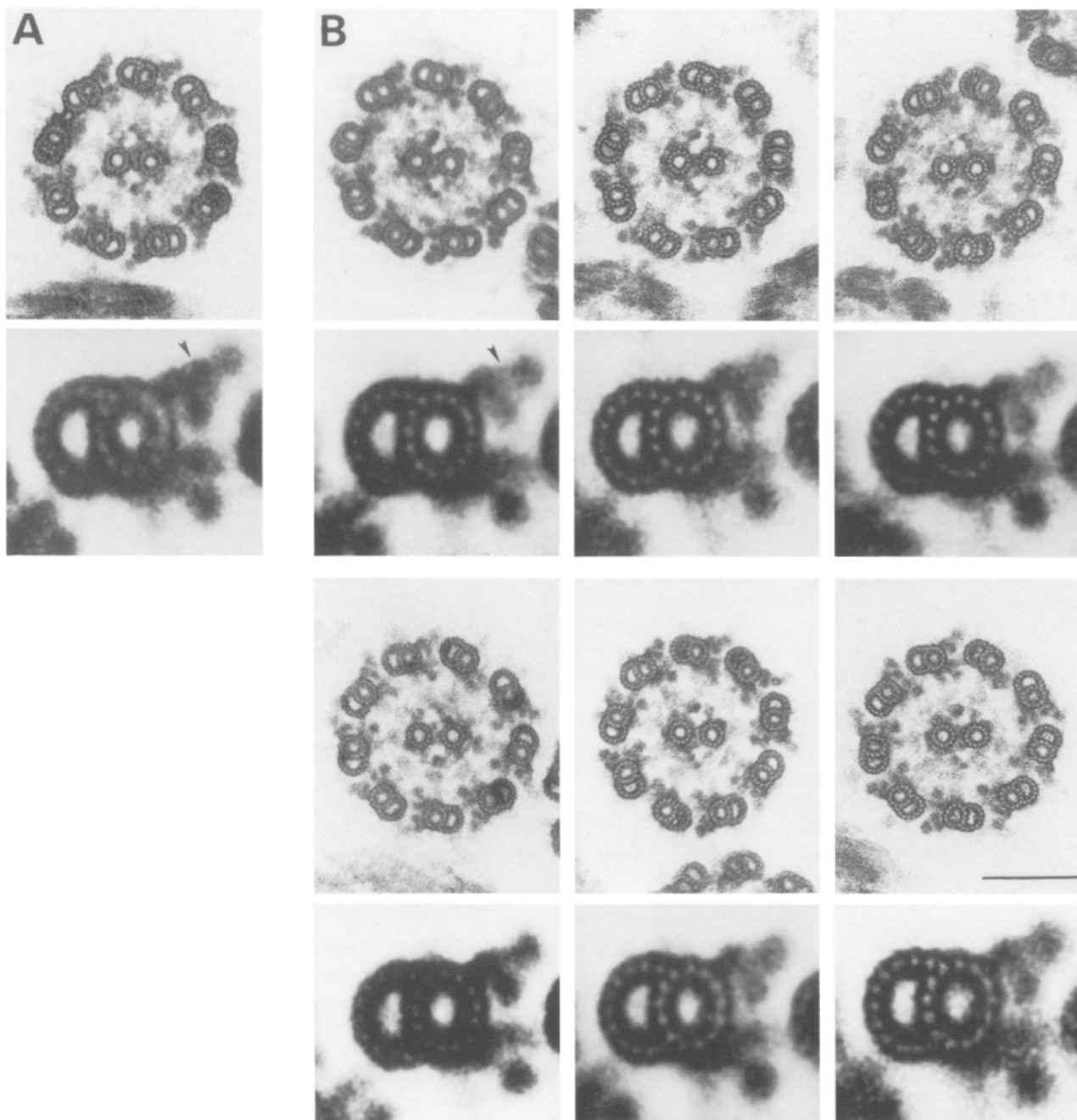


Figure 8. Electron micrographs of cross sections of wild-type and *oda4-s7* axonemes with averaged outer-doublet images from these axonemes. (A) wild type; (B) *oda4-s7*. The micrographs of *oda4-s7* shown are examples that have an almost complete number (i.e., eight) of outer arms. Arrowheads, sites where difference can be seen between wild type and mutant. Bar, 0.1 μm .

pearance. Previously, we found that the α heavy chain forms the outermost appendage of the outer arm (Sakakibara et al., 1991). The double mutant *oda4-s7xoda11* retains the innermost lobe of the arm, which probably corresponds to the γ heavy chain. These observations define the approximate location of all three dynein heavy chains in the cross section image of the outer arm (Fig. 11).

The present finding that the *oda4-s7* mutant expressing a truncated β heavy chain retains the outer arm, whereas the previously described *oda4* mutant does not, strongly suggests that the NH_2 -terminal portion of the β heavy chain is

important in assembly of the outer arm or in attaching the outer arm to the doublet. The NH_2 -terminal region of the β heavy chain associates directly with the 69-kD intermediate chain (King and Witman, 1989; Mitchell and Rosenbaum, 1986), which is a component of a discrete intermediate chain-light chain complex located at the base of the soluble dynein (King and Witman, 1990). Another component of this complex, the 78-kD intermediate chain, is in direct contact with the α tubulin in the axoneme and may be involved in binding the arm to the outer doublet (King et al., 1991). Therefore, loss of any one of these three components (the β

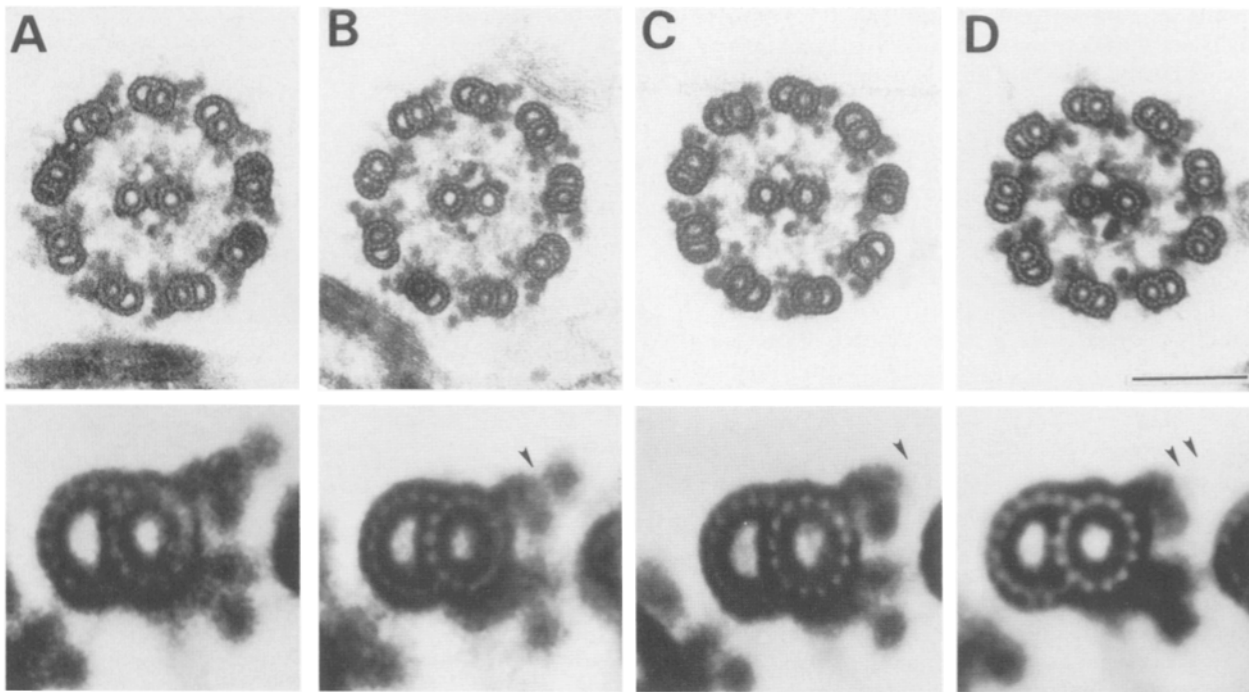


Figure 9. Comparison of shape of outer dynein arms in cross sections of axonemes of (A) wild type; (B) *oda4-s7*; (C) *oda11*; and (D) *oda4-s7xoda11*. Bottom row shows averaged images. Averaging was performed with either eight (A, B, and C) or five outer doublets (D) that have attached outer arms. Arrowheads, portions missing from the mutant arms. Any minor variation in the inner arm images between different strains is probably due to a variation in the quality of electron micrographs. Bar, 0.1 μm .

heavy chain, the 69-kD intermediate chain, or the 78-kD intermediate chain) might prevent either assembly of the outer arm, or attachment of a preassembled arm to the outer doublet. Indeed, mutations in the 69-kD intermediate chain

(*oda6*; Mitchell and Kang, 1991) result in the disappearance of the entire outer arm (Kamiya, 1988), and the gene encoding the 78-kD intermediate chain maps at or near the *oda9* mutation (King, S. M., C. G. Wilkerson, and G. B. Witman, unpublished results), which also results in loss of the outer arm (Kamiya, 1988). There is no evidence that the β heavy chain itself binds directly to the A-tubule.

Function of the β Heavy Chain

The swimming velocity and flagellar beat frequency in

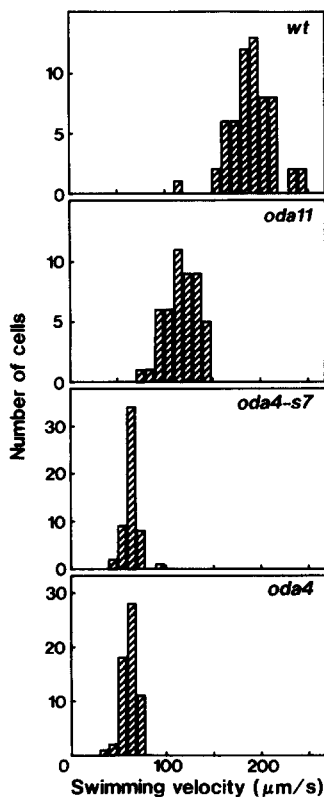


Figure 10. Swimming velocities of live cells of wild type, *oda11*, *oda4-s7*, and *oda4* ($n = 50$). The *oda4-s7* cells used had an average (SD) of 5.5 (1.2) outer arms in axoneme cross section in 30 electron micrographs. Temperature: 25°C. The average velocity and standard deviation for each strain is given in the text.

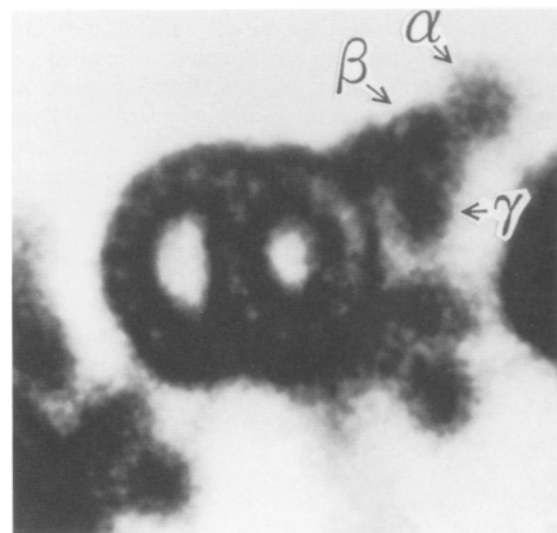


Figure 11. Probable locations of three outer arm heavy chains (α , β , and γ) in the wild-type outer arm.

oda4-s7 cells were only slightly higher than those of *oda4* cells, which lack the entire outer arm. This indicates that the outer arms in *oda4-s7* function only very poorly. Although the number of outer arms itself is reduced in this mutant to ~70% of the wild-type level, its poor motility should not result solely from the reduction in the number of outer arms, because we have previously shown that the axonemal motility in *oda* mutants increases upon addition of the outer arms and that the increase is approximately linear with the number of outer arms attached (Sakakibara and Kamiya, 1989; Takada et al., 1992). Hence, it is likely that the great reduction in motility is caused mostly by the absence of the functional β heavy chain. This situation differs from that of *oda11*, where absence of the α heavy chain results in only a partial reduction of swimming velocity and flagellar beat frequency.

The β heavy chain thus apparently plays a more important role in outer-arm function than does the α heavy chain. Indeed, the β heavy chain may be the major force-producing subunit in the outer arm of *Chlamydomonas*, as has been suggested for the β heavy chain of sea urchin sperm outer arm dynein (Moss et al., 1992b). The α heavy chain may be an amplifier of the function of the β heavy chain (and possibly of the γ heavy chain) and unable to function by itself. Although the exact mechanism by which the three heavy chains cooperate to function as an outer dynein arm must await further studies, the present study strongly suggests that the α and β heavy chains differ greatly in their functional properties. For a further understanding of the function of each outer arm heavy chain, it will be important to examine their motor properties using an in vitro motility assay system (Paschal et al., 1987; Vale and Toyoshima, 1989; Sale and Fox, 1988; Moss et al., 1992a,b; Kagami and Kamiya, 1992). It will be particularly interesting to determine whether the functional difference between different heavy chains observed in mutants is correlated with any striking difference in in vitro properties, as demonstrated with the α and β heavy chains in sea urchin sperm outer arm dynein (Sale and Fox, 1988; Moss et al., 1992a,b).

The present study has revealed a significant functional difference between the α and β outer arm heavy chain of *Chlamydomonas*. The function performed by the third, γ , heavy chain remains to be determined; indeed, it is not known whether the outer arm can function without the γ heavy chain. We currently are trying to answer this question by isolating new mutants that retain the outer arm but have a defect in the γ heavy chain, as well as by combining purified α and β heavy chains with *oda* axonemes in an in vitro system (Takada et al., 1992).

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